

The effect of a tin-containing fluoride mouth rinse on the bond between resin composite and erosively demineralised dentin

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The effect of a tin-containing fluoride mouth rinse on the bond between resin composite and erosively demineralised dentin

Abstract

Objectives: To evaluate the effect of a tin-containing fluoride (Sn/F) mouth rinse on microtensile bond strength (μ TBS) between resin composite and erosively demineralised dentin.

Materials and Methods: Dentin of 120 human molars was erosively demineralised using a 10-day cyclic de- and remineralisation model. For 40 molars the model comprised erosive demineralisation only, for another 40 the model included treatment with NaF solution, and for yet another 40 the model included treatment with a Sn/F mouth rinse. In half of these molars ($n=20$) the demineralised organic matrix was continuously removed by collagenase. SiC paper ground, non-erosively demineralised molars served as control ($n=20$). Subsequently, μ TBS of Clearfil SE/Filtek Z250 to the dentin was measured and failure mode determined. Additionally, surfaces were evaluated by SEM and EDX.

Results: Compared to the non-erosively demineralised control, erosive demineralisation resulted in significantly lower μ TBS regardless of the removal of demineralised organic matrix. Treatment with NaF increased μ TBS, but the level of μ TBS obtained by the non-erosively demineralised control was only reached when the demineralised organic matrix had been removed. The Sn/F mouth rinse led to significantly higher μ TBS than did the non-erosively demineralised control, both with or without removal of demineralised organic matrix. The Sn/F mouth rinse yielded higher μ TBS than did the NaF solution.

Conclusions: Treatment of erosively demineralised dentin with a NaF solution or a Sn/F mouth rinse increased the bond strength of resin composite.

Clinical Relevance: Bond strength of resin composite to eroded dentin was not negatively influenced by treatment with a tin-containing fluoride mouth rinse.

Keywords

Erosion • Sodium fluoride • Stannous chloride • Collagenase • Adhesion

Introduction

In case of severe dental erosion (e.g. caused by eating disorders with chronic vomiting) symptomatic treatment is often indicated. One approach aims to prevent further loss of tooth substance by forming acid resistant mineral precipitations. Fluoride preparations containing monovalent or organic fluoride compounds may cause precipitation of calcium fluoride-like deposits on the tooth surface. However, these deposits probably offer limited protection against acid impacts as they readily dissolve at acidic pH [1]. In contrast, promising effects have been demonstrated with fluoride compounds containing polyvalent metal cations such as titanium fluoride [2] or stannous fluorides [3,4]. The combination of stannous chloride and fluoride has been shown to be particularly effective against erosive mineral loss in enamel and dentin [5].

On enamel, the application of tin-containing fluoride (Sn/F) solutions may result in the formation of various mineral salts such as $\text{Sn}_2(\text{PO}_4)\text{OH}$, $\text{Sn}_3\text{F}_3\text{PO}_4$, and $\text{Ca}(\text{SnF}_3)_2$ [6-8], but little is known about interactions with dentin. Element analyses have shown that tin is deposited on dentin surfaces especially under erosive conditions and is incorporated both in the demineralised and mineralised tissue [9].

Although loss of tooth substance by erosion can be inhibited by appropriate non-invasive strategies, it is often necessary to restore the structural integrity and/or to reduce hypersensitivity of eroded teeth with resin composites. A 3-year randomized clinical study, however, has shown a higher failure rate of direct and indirect resin composite restorations in patients with tooth wear due to dental erosion than in patients without [10]. One possible explanation for the increased failure of these restorations is impaired adhesion of the resin composite to eroded dentin as compared to sound dentin, but studies on the issue are lacking. In addition, the

influence of fluoride applications on bond strength of resin composites to eroded dentin has not been investigated so far. Particularly Sn/F solutions are of interest as they alter the composition and solubility of dentin mineral, which could affect the properties of dentin as bonding substrate, and are currently among the most effective available products for daily home use.

The aim of this study was therefore to investigate the microtensile bond strength (μ TBS) between resin composite and non-eroded dentin, erosively demineralised dentin, or erosively demineralised dentin subjected to a NaF solution or to a Sn/F mouth rinse. To mimic eroded dentin with and without a layer of demineralised organic matrix, dentin was eroded according to two different protocols: without or with continuous removal of demineralised organic matrix by use of collagenase. The null hypothesis was that there would be no differences in μ TBS-values between all groups.

Additionally, the dentin surfaces of all groups were qualitatively evaluated by scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX).

Materials and methods

Preparation of dentin specimens

Sound, extracted human third molars were cleaned under tap water and stored in 0.5% chloramin solution at 4°C until use. Then, the molars were embedded in circular molds with self-curing acrylic resin (Paladur, Heraeus Kulzer GmbH, Hanau, Germany) and ground with a grinding machine and silicon carbide (SiC) papers grit #320 (Struers LaboPol-21/SiC #320, Struers, Ballerup, Denmark) until the entire surface was in coronal dentin. The dentin surfaces were air-dried and carefully checked for absence of enamel. Molars exhibiting exposure of the dental pulp were

discarded. Finally, all molars were ground for 15 s with SiC papers grit #500 (Struers LaboPol-21/SiC #500, Struers), using a new SiC paper after the grinding of ten molars.

A final amount of 161 extracted human third molars was used: Twenty molars for μ TBS measurements and three molars for qualitative SEM and EDX evaluation were left untreated and served as non-erosively demineralised control (Group 1). Twenty molars for μ TBS measurements and three molars for qualitative SEM and EDX evaluation of dentin surfaces were randomly allocated to each of the six groups of erosive demineralisation without and with continuous removal of demineralised organic matrix (Groups 2a/b, 3a/b, 4a/b).

Erosive demineralisation

For erosive demineralisation, molars of Groups 2a/b, 3a/b, and 4a/b were subjected to a cyclic de- and remineralisation procedure over a total of 10 days (5 days/week; two weeks). Molars were erosively demineralised with 0.05 M citric acid solution (pH 2.3, anhydrous citric acid, Merck, Darmstadt, Germany) six times per day for 5 min under agitation in a shaking bath (30/min, 25°C; GFL Model 1083, Burgwedel, Germany). After the first erosive demineralisation and between the following erosive demineralisations, molars were stored in a remineralisation solution for 60 min (shaking bath; 30/min, 25°C; GFL Model 1083) according to Gerrard & Winter (4.08 mM H_3PO_4 , 20.10 mM KCl, 11.90 mM Na_2CO_3 , and 1.98 mM CaCl_2 with a pH of 6.7 (all chemicals from Merck)) [11]. For continuous removal of demineralised organic matrix (Groups 2b, 3b, 4b), 100 units collagenase/ml were added to the remineralisation solution (pH 7.4; Collagenase from *Clostridium histolyticum* type VII with a collagen removal activity of 1.680 units/ μg , solid at 25°C at pH 7.5 in the presence of calcium ions; Sigma Aldrich, St. Louis, MO, USA). Over the weekend,

molars were stored in a humid chamber (100% relative humidity) at 4°C. Solutions were renewed at the beginning of each experimental day. Groups 2a/b underwent erosive demineralisation only. In Groups 3a/b a NaF solution (NaF 500 ppm, adjusted to pH 4.5 with 1% HCl) and in Groups 4a/b a Sn/F mouth rinse (125 ppm F as amine fluoride (AmF), 375 ppm F as NaF, and 800 ppm Sn as SnCl₂, pH 4.5; Elmex® erosion protection, GABA International, Therwil, Switzerland) was used for 2 min after the first and the last demineralisation period. Before transfer to a new solution, molars were rinsed for 1 min with tap water.

Microtensile bond strength (μ TBS) measurements and failure mode determination

Subsequently, the two bottle, self-etch adhesive system Clearfil SE (Kuraray, Okayama, Japan) was applied on the dentin surface of the 20 molars in each group according to manufacturer's instructions (*Primer*: 10-methacryloyloxydecyl dihydrogen phosphate (MDP), 2-hydroxyethyl methacrylate (HEMA), hydrophilic dimethacrylate, dl-camphorquinone, water; Lot No: 00965AA - *Bond*: MDP, HEMA, bis-phenol A diglycidylmethacrylate (Bis-GMA), hydrophobic dimethacrylate, dl-camphorquinone, silanated colloidal silica; Lot No: 01387AA). After adhesive treatment, the molars were built up in two layers of 2 mm each with a resin composite (Filtek Z250, 3M ESPE, St. Paul, MN, USA; shade A4, Lot No: N183899). Each layer of resin composite was light-cured for 20 s and all light-curing was performed with an LED light-curing unit (Demi, Kerr Corporation, Middleton, WI, USA). Light power density was verified to be at least 1000 mW/cm² at the beginning and end of each day of specimen preparation with a radiometer (Demetron LED Radiometer, Kerr Corporation). The molars with the resin composite build-ups were stored for 24 h in 100% humidity at 37°C.

After storage, the molars were sectioned with an electronically programmable diamond saw under water-cooling (Struers Accutom-5 with Struers Diamond Cut-Off Wheel 330CA, Struers) perpendicularly to the adhesive interface in both x and y directions to obtain nine sticks from the most central part of each molar. Four sticks per molar were randomly selected for measurement of μ TBS. In the rare cases of pretesting failure (13 pretesting failures/560 final sticks), one of the remaining five sticks of the same tooth was used as replacement. In order to monitor the size and to calculate the bonding surface (BSU (mm^2)) of each stick, width and breadth of the sticks were measured with a digital caliper (Mitutoyo IP 65, Kawasaki, Japan; mean width (standard deviation): 1.05 (0.03) mm, mean breadth (standard deviation): 1.05 (0.02) mm). Then, the sticks were fixed by their ends to notched Ciucchi's jigs mounted in a universal testing machine (Syndicad TC-550, Syndicad Dental Research, Munich, Germany) with a low viscosity resin (Heliobond, Ivoclar Vivadent AG, Schaan, Liechtenstein). The sticks were stressed in tension at a crosshead speed of 1.0 mm/min until fracture and the maximum force (F_{max} (N)) was recorded. The μ TBS-values (MPa) were calculated according to the formula $\mu\text{TBS} = F_{\text{max}} / \text{BSU}$.

Additionally, the failure mode of each stick was stereomicroscopically determined at 45 \times magnification (Leica ZOOM 2000, Leica, Buffalo, NY, USA) and classified into one of the five following categories: 1) cohesive failure in *dentin*, 2) adhesive failure between *adhesive system* and *dentin*, 3) mixed *adhesive* failure (failure modes 2 and 4), 4) adhesive failure between *adhesive system* and *resin composite*, and 5) cohesive failure in *resin composite*.

Qualitative SEM and EDX evaluation of dentin surfaces

Three molars from each group were mounted on aluminium stubs and dehydrated in a desiccator for five days and then sputter-coated with gold/palladium (100 s, 50 mA) using a sputtering device (Balzers SCD 050, Balzers, Liechtenstein). Dentin surfaces were evaluated under an SEM (JSM-6510, Jeol, Tokyo, Japan) and micrographs of 5000× magnification were made.

For EDX, the acceleration voltage of the SEM was set to 15 kV and EDX spectra were collected using a Silicon Drift Droplet Detector (X-Flash Detector 410-M, Bruker Nano GmbH, Berlin, Germany). Count rates were ~1 kcps and remained constant during the measurements, indicating that neither contamination nor loss of mass affected the results.

Statistical Analysis of μ TBS-values

Out of the four μ TBS-values obtained per molar, a mean μ TBS-value was calculated. Therefore, 20 μ TBS-values per group were used for statistical analysis with a nonparametrical ANOVA model for two fixed factors according to Brunner & Munzel [12] followed by Wilcoxon rank sum tests and Bonferroni-Holm adjustment for multiple testing. The main statistical analysis was performed with R version 2.9.1 (The R Foundation for Statistical Computing, Vienna, Austria; www.R-project.org).

The μ TBS-data of preliminary tests had been statistically analysed with NCSS/PASS 2005 (NCSS, Kaysville, UT, USA) for sample size determination after the level of significance had been set at $\alpha=0.05$.

Results

Microtensile bond strength (μ TBS) measurements and failure mode determination

The μ TBS-values are shown in Figure 1 and p-values of group comparisons are given in Table 1. The nonparametrical ANOVA model showed significant differences between the groups ($p < 0.0001$) and a significant effect of continuous removal of demineralised organic matrix ($p < 0.0001$) with significantly higher μ TBS-values when the demineralised organic matrix had been removed. No statistically significant interaction was found between continuous removal of demineralised organic matrix and the different groups, indicating that continuous removal of demineralised organic matrix had the same effect on μ TBS-values in Groups 2, 3, and 4.

Compared to the non-erosively demineralised control (Group 1), the erosive demineralisation resulted in significantly lower μ TBS-values regardless of the removal of demineralised organic matrix (Groups 2a/b). Treatment with NaF (Groups 3a/b) increased μ TBS-values, but the μ TBS-values of the non-erosively demineralised control was only reached when the demineralised organic matrix had been removed (Group 3b). In contrast, the application of the Sn/F mouth rinse (Groups 4a/b) led to significantly higher μ TBS-values compared to the non-erosively demineralised control (Group 1), both with and without removal of demineralised organic matrix. Compared to erosive demineralisation only (Groups 2a/b), treatment with both NaF solution (Groups 3a/b) and Sn/F mouth rinse (Groups 4a/b) increased μ TBS-values regardless of the removal of demineralised organic matrix, the Sn/F mouth rinse yielding higher μ TBS-values than the NaF solution.

The distribution of failure modes is shown in Table 2. The non-erosively demineralised control (Group 1) presented a majority of mixed adhesive failures followed by adhesive failures between adhesive system and dentin. The predominant failure mode of Groups 2a/b and 3a was adhesive failure between adhesive system

and dentin. In Group 3b and Groups 4a/b the predominant failure mode was mixed adhesive failure. Continuous removal of demineralised organic matrix (Groups 2b, 3b, and 4b) showed an increased amount of both mixed adhesive failures and adhesive failures between adhesive system and resin composite when compared to Groups 2a, 3a, and 4a (without continuous removal of demineralised organic matrix).

Qualitative SEM and EDX evaluation of dentin surfaces

Qualitative SEM evaluation revealed quite a similar dentin surface for all three molars in each group. Representative micrographs are shown in Figure 2.

The non-erosively demineralised dentin surfaces showed typical signs of a smear layer with parallel undulations caused by grinding with no visible tubules (Figure 2A). In the groups with erosive demineralisation, the smear layer had been entirely removed and open tubules were apparent on all surfaces (Figure 2 B-G). The peritubular dentin was absent except after treatment with Sn/F mouth rinse and continuous removal of the demineralised organic matrix (Group 4b), and the intertubular areas appeared more or less amorphous with no obvious differences between groups.

The EDX spectra revealed distinct differences between groups treated with or without continuous removal of demineralised organic matrix. In Groups 2a, 3a, 4a without continuous removal of demineralised organic matrix, the dentin surface was fully demineralised with only small peaks for Ca and P (Figure 3 B, D, F). In contrast, in Groups 2b, 3b, 4b with continuous removal of demineralised organic matrix, much more mineral with distinct peaks for Ca and P was present (Figure 3 C, E, G). In Groups 4a/b (treated with the Sn/F mouth rinse), tin was clearly detected in Group 4b with continuous removal of demineralised organic matrix, but not in Group 4a without continuous removal of demineralised organic matrix.

Discussion

Clinically, restoring eroded teeth is particularly indicated in cases with severe loss of tooth substance. The present study aimed to simulate such clinical conditions by creating severe erosive demineralisation as described in previous studies [13,14]. In vitro erosive demineralisation with acids (of clinically relevant amounts and time durations) results in a dissolution of mineral with persisting organic matrix [15]. With the model of cyclic de- and remineralisation used in the present study, loss of mineral in the order of 90 μm occurs [16], thus resulting in a broad zone of demineralised organic matrix on the dentin surfaces. These dentin surfaces appear wet, soft and resilient - a pattern which is not observed clinically. Knowledge about the structure of eroded dentin in vivo is sparse, but it can be assumed that proteolytic intraoral enzymes continuously remove demineralised dentin from the surface. As it is not clear how fast and how complete these proteolytic effects are, dentin in the present study was eroded according to two different protocols: without or with continuous removal of demineralised organic matrix by use of collagenase, which has previously been shown to be effective [14].

Adhesion of resin composite to dentin depends on the interaction of the adhesive system and the dentin surface and depends on the ability of the adhesive system (mainly of the primer) to penetrate dentin including the formation of a future hybrid layer. The adhesion mechanism is assumed to be predominantly micromechanical, but also physicochemical interactions are possible [17]. The adhesive system used in the present study (Clearfil SE) is a two bottle, self-etch adhesive system and can be considered as well-investigated and well-documented [18,19]. The fact that with the Clearfil SE adhesive system 1) phosphoric acid-etching is not performed, and 2) Primer and Bond are applied without extensive manipulation (e.g. by "brushing in" of the primer) might be of advantage because phosphoric acid-etching and "brushing in"

of the primer might alter the structure of erosively demineralised dentin and its organic matrix uncontrollably. Clearfil SE contains an acidic phosphate monomer (MDP), which has been demonstrated to be capable of bonding to hydroxyapatite (HAP) [20], leaving the option for not only micromechanical but also chemical bonding to tooth substance. Proposed interaction of MDP to HAP is ionic bonding of phosphate groups to calcium or covalent bonding via condensation with the PO_4^{3-} ions of HAP. The MDP monomers are adsorbed to HAP inducing the dissolution of calcium and phosphate followed by the deposition of MDP-calcium salts and calcium phosphate [21]. MDP dissolves calcium from HAP and interacts as calcium salt with dentin structures. A study investigating the intermolecular interaction of MDP and its calcium salt with collagen revealed that both forms might have different conformational states and docking sites [22] both contributing to the bonding of MDP. Normally, adhesive systems are applied on sound dentin covered with a smear layer or on sclerotic dentin (e.g. of wedge-shaped defects). In the present study, however, the substrate for bonding was erosively demineralised dentin with the organic matrix being demineralised to various degrees as confirmed by EDX. Thus, the question arises how the interaction between Clearfil SE and the respective substrates is characterised.

Regarding the fully demineralised organic matrix, it is well conceivable that due to the hydrophilic properties of MDP the primer penetrates quite deeply into the organic matrix. It is, however also reasonable to assume that the penetration is incomplete and that the bond strength values observed represent the tensile strength of the adhesive system to the remaining, unpenetrated demineralised dentin. Thus, it is not surprising that the bond strength values were significantly lower than to the non-erosively demineralised control. After treatment with the NaF solution significantly higher bond strength values were found. It has been shown that a 500 ppm NaF

solution reduced the erosive mineral loss in dentin by about 40% [23]. This suggests that in the present study, as well, the demineralised organic matrix was much thinner after NaF treatment than after erosive demineralisation only. Consequently, the penetration of the adhesive system may be more advanced thus increasing bond strength. With the Sn/F mouth rinse a reduction of erosive mineral loss by about 50% can be expected [23], resulting in a thickness of the demineralised organic matrix similar to that obtained after treatment with the NaF solution. Interestingly, however, the bond strength values were markedly higher after Sn/F treatment than after application of NaF. This may be explained by the fact that the organic matrix, though fully demineralised, retains tin to a certain extent [14]. Tin ions could occupy negatively charged bonding sites such as phosphate groups in the organic matrix, thus making the collagen structure less polar and more accessible for the adhesive. Also, tin ions could function as calcium in the MDP-calcium salt and promote "docking" of MDP to collagen. Finally, a further mode of action is conceivable: Within the organic matrix and its collagen network, proteoglycans function as regulators for the three-dimensional arrangement of the collagen network and for the spacing of the collagen fibrils [24]. The removal of proteoglycans has been shown to enlarge spaces between collagen fibrils and to increase bond strength values [25] and the authors speculated that these empty intercollagen spaces are more accessible to the adhesive. Proteoglycans can be removed by enzymes [25] but also by acids at low pH [24] or relatively highly concentrated MgCl (0.5 M) [24]. It can be speculated that the Sn/F mouth rinse containing SnCl₂ exhibits similar effects resulting in an enhanced infiltration of the adhesive system.

In the groups with continuous removal of the demineralised organic matrix the dentin surfaces were much more mineralised. The SEM micrographs and EDX spectra revealed more or less fully mineralised intertubular dentin with open tubules. These

surfaces might approximate the clinical structure of active erosive lesions under severe dental erosion (e.g. caused by eating disorders with chronic vomiting) and the potential continuous removal of organic matrix by the multitude of salivary [26] or gastric proteolytic enzymes [27]. In all groups with continuous removal of demineralised organic matrix bond strength values were higher than in the groups without removal.

In the erosively demineralised groups without any fluoride treatment bond strength values did not reach those of the non-erosively demineralised control although the degree of mineralisation was similar. It is well conceivable that the removal of collagen under the severe erosive conditions used in the present study was not complete, leaving amorphous remnants of organic material on the surface. This is supported by the finding that in two thirds of the molars, the failure mode was adhesive failure between adhesive system and dentin. After treatment with the NaF solution, bond strength values were similar or higher to those of the erosively demineralised controls. This might be due to a more complete removal of the demineralised organic matrix with slower erosive demineralisation when treated with the NaF solution. The treatment with the Sn/F mouth rinse resulted in the highest bond strength values. It has been shown that tin is retained on and in dentin after application of Sn/F solutions and continuous removal of demineralised organic matrix [14], which was confirmed in the present study. The exact erosion protective mechanism of tin is unknown, but it can be speculated that various forms of Sn/F salts make the remaining mineral acid resistant. This speculation is confirmed by the SEM micrograph of the dentin surface after treatment with Sn/F mouth rinse and continuous removal of the demineralised organic matrix, in that peritubular dentin is clearly visible indicating an overall higher mineral content. Whether the high bond strength values are due to the amount of mineral or due to adhesion to tin-supported

HAP being superior than adhesion to HAP alone is subject for further research. Finally, some of the discussed adhesion mechanisms and interactions may only be valid for the MDP-containing adhesive system Clearfil SE. At this point, it therefore remains to be investigated how other adhesive systems interact with eroded dentin as well as with eroded dentin treated with NaF or Sn/F solutions and mouth rinses.

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Conflicts of interest

The authors declare no conflicts of interest, real or perceived, financial or nonfinancial.

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Captions to Figures

Figure, SEM micrographs, and EDX spectra

File: Figure1.eps

Fig. 1 Microtensile bond strength values of the different groups (median, lower/1st and upper/3rd quartile, and minima/maxima) - a: no continuous removal of demineralised organic matrix; b: continuous removal of demineralised organic matrix

File: Figure2.tiff

Fig. 2 Micrographs of the scanning electron microscopy (SEM) for qualitative evaluation of dentin surfaces (Groups a: no continuous removal of demineralised organic matrix; Groups b: continuous removal of demineralised organic matrix)

File: Figure3.tiff

Fig. 3 Spectra of the energy dispersive X-ray spectroscopy (EDX) for qualitative evaluation of dentin surfaces (Groups a: no continuous removal of demineralised organic matrix; Groups b: continuous removal of demineralised organic matrix)

Table 1 Comparison of microtensile bond strength values by Wilcoxon rank sum tests: p-values marked with * show significant differences between groups after Bonferroni-Holm adjustment for multiple testing

	Group 2a (erosive demineralisation)	Group 2b (erosive demineralisation)	Group 3a (erosive demineralisation, NaF solution)	Group 3b (erosive demineralisation, NaF solution)	Group 4a (erosive demineralisation, Sn/F mouth rinse)	Group 4b (erosive demineralisation, Sn/F mouth rinse)
Group 1 (non-erosively de- mineralised control)	<0.0001*	<0.0001*	0.0002*	0.8498	0.4652	<0.0001*
Group 2a	-	0.0018*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
Group 2b	-	-	0.0012*	<0.0001*	<0.0001*	<0.0001*
Group 3a	-	-	-	0.0030*	0.0022*	<0.0001*
Group 3b	-	-	-	-	0.3834	<0.0001*
Group 4a	-	-	-	-	-	0.0004*

Groups 2a, 3a, 4a: no continuous removal of demineralised organic matrix; Groups 2b, 3b, 4b: continuous removal of demineralised organic matrix

Table 2 Failure mode determination

Failure mode (80 sticks, 4 per molar)	1) Cohesive failure in <i>dentin</i>	2) Adhesive failure between <i>adhesive</i> <i>system and</i> <i>dentin</i>	3) Mixed <i>adhesive</i> failure (failure modes 2 and 4)	4) Adhesive failure between <i>adhesive</i> <i>system and</i> <i>resin composite</i>	5) Cohesive failure in <i>resin</i> <i>composite</i>
Groups (n=20 molars per group)					
Group 1 (non-erosively demineralised control)	1%	35%	59%	5%	0%
Group 2a (erosive demineralisation)	0%	83.%	17%	0%	0%
Group 2b (erosive demineralisation)	0%	63%	36%	1%	0%
Group 3a (erosive demineralisation, NaF solution)	0%	54%	46%	0%	0%
Group 3b (erosive demineralisation, NaF solution)	0%	34%	54%	12%	0%
Group 4a (erosive demineralisation, Sn/F mouth rinse)	1%	34%	50%	14%	1%
Group 4b (erosive demineralisation, Sn/F mouth rinse)	0%	25%	59%	15%	1%

Groups 2a, 3a, 4a: no continuous removal of demineralised organic matrix; Groups 2b, 3b, 4b: continuous removal of demineralised organic matrix





